

### **REMARKS**

Applicants have received and reviewed an Office Action dated December 18, 2006. By way of response, Applicants have amended claims 1, 7, 25, and 26. No new matter has been added. Claims 1-3, 5-9, 12-14, 17-19, and 23-28 are pending. Applicants submit that the pending claims are supported by the specification.

For the reasons given below, Applicants submit that the pending claims are in condition for allowance, and notification to that effect is earnestly solicited.

### **Claim Objections**

The Examiner objected to claim 7 on the basis that the word "containing" in line 3 of the claim should have been struck through to indicate deletion. Claim 7 has been amended accordingly.

### **Claim Rejections – 35 USC § 112**

The Examiner rejected claims 1 and 25 on the basis that it is unclear what form or manner the "detection of a change" encompasses. It should be noted that a person of skill in the art would very well know what an aldehyde detecting reagent does and the resulting changes that can be monitored to monitor the presence of aldehydes. The change can be any change that is detectable or measurable by having the aldehyde detecting reagent detect available aldehydes. Applicant should not be required to limit the claims to a particular type of detectable change.

The Examiner rejected claims 25 and 26 on the basis that it is unclear whether it is meant that an enzyme is not present. Please note that claim 25 was inserted to mean that an enzyme is not required to modify the cancer marker to enable detection by Schiff's reagent. Claim 25 has been amended to clarify this. Claim 26 specifies that the enzyme not present is galactose oxidase and optionally catalase. Claim 26 was amended to depend on claim 1 to clarify this.

Accordingly, Applicants respectfully submit that the amended claims fully comply with § 112, second paragraph, and withdrawal of this rejection is respectfully requested.

**Claim Rejections – 35 USC § 102**

The Examiner rejected claim 1 as being anticipated by Shamsuddin (US 5,348,860). Applicants respectfully traverse this rejection.

The Examiner asserted that Shamsuddin teaches a method of screening for a cancerous or precancerous condition including adsorbing a sample of proteinaceous secretion associated with the organ on to a protein capturing water insoluble substrate, and treating the sample with an aldehyde detecting reagent (basic fuchsin) to visually detect marker carbohydrates.

It is respectfully submitted that this description of Shamsuddin is not completely accurate and there are in fact several differences between the present invention and that of Shamsuddin.

Claim 1 of the present invention as amended is directed to a method for detecting neoplasia, a precancerous condition, or cancer of the breast in a subject including treating a sample of breast fluid from the subject with an aldehyde detecting reagent without any prewashing wherein the sample is suspected of containing an aldehyde marker associated with breast cancer the detection of a change produced by the aldehyde detecting reagent compared to a control is indicative of the presence of the aldehyde marker in the sample which is indicative of neoplasia, a precancerous condition, or cancer.

Basis for this can be found in numerous passages throughout the specification, for example at page 9, lines 24-32. The claim therefore includes the condition that the sample of breast fluid is treated with an aldehyde detecting reagent without any prewashing and that the sample is suspected of containing an aldehyde marker associated with breast cancer.

Shamsuddin does not teach or suggest how to determine aldehydes in Nipple Aspirate Fluid (NAF). Shamsiddun teaches how to detect presence of Thomsen-Friedenreich antigen (TF-antigen) and related glycoproteins, in various body fluids. TF-antigen is a galactose-containing glycoprotein which has been shown to be related to colorectal cancer several decades ago. Shamsuddin does not teach that any aldehyde is a marker of breast cancer. The most Shamsuddin would teach is that Thomsen-Friedenreich antigen, a glycoprotein not an aldehyde, is a cancer marker.

Shamsuddin teaches a method for detection of Thomsen-Friedenreich Antigen which consists of the following steps:

- a) oxidation of OH – 6 (hydroxyl in the position 6) on galactose in TF-antigen by the enzyme galactose oxidase using commercial preparations of the enzyme (containing the enzyme catalase used to deactivate hydrogen peroxide formed as by-product);
- b) detection of this modified TF-antigen by treating NAF with Schiff reagent that is well known to produce purple-magenta coloration with aldehydes.

As such, there are, in fact, several differences between the method of the present invention and that of Shamsuddin. In order to emphasize these differences, claim 1 has been amended to incorporate the limitation that the sample is suspected of containing an aldehyde marker associated with breast cancer. This is certainly not the case with Shamsuddin, where the method is intended to detect a saccharide marker (column 3, lines 35-36, column 4, line 10, column 5, lines 62-63).

Shamsuddin states (claim 1) that he detects a glycoprotein marker. He claims the use of two oxidizing agents: one, which results in oxidative carbohydrate ring opening not using galactose oxidase (claim 1) or selectively oxidizing the primary hydroxyl group of the galactose sugar moiety (further claim 10) by galactose oxidase (claims 11,20). It should be noted that through the claims and the body of the patent Shamsuddin uses the term carbohydrate often but it invariably means a carbohydrate moiety of a glycoprotein as it is impossible to capture a carbohydrate by protein-capturing substrate before the oxidation; the oxidative method used is that using galactose oxidase. From claims 19, 20, and 23 of Shamsuddin, it appears that the oxidation with galactose oxidase is used to detect specifically the carbohydrate portion of TF-antigen and related glycoproteins, while the oxidative ring opening with periodic acid is used to detect generally the presence of any carbohydrate in the discharge.

For this reason, the method of Samsuddin, as noted above is at least a two step procedure which requires a first step in which the sample is treated with an oxidizing agent (galactose oxidase), which results in oxidative ring opening of any saccharide present in the sample at a hydroxy group bearing ring carbon atom to form an aldehydic sugar moiety (column 3, lines 35-38). The oxidized sample is then reacted with Schiff's reagent.

The oxidation step of Shamsuddin is essential because Schiff's reagent does not detect saccharides and the presently claimed method differs from that of Shamsuddin

because a sample of breast fluid is treated with aldehyde detecting agent. In Shamsuddin, it is not a sample of breast fluid which is treated with an aldehyde detection agent as the authors of Shamsuddin were assaying for saccharides. Rather if one were to practice the teachings of Shamsuddin, it would be an oxidized sample of breast fluid that would be treated with the aldehyde detection agent (column 3, lines 39-40, column 6, line 24 etc).

The detection of an aldehyde marker is neither taught nor suggested by the Shamsuddin reference and a person of skill in the art would not have concluded from the teaching of Shamsuddin that it was possible to omit the oxidizing step. Further it should be noted that there is no example in Shamsuddin for the detection of breast cancer.

Secondly, claim 1 has been amended to state that there is no pre-washing step and the lack of a prewashing step also distinguishes the present invention from the method of Shamsuddin. Shamsuddin uses prewashing but the present invention surprisingly teaches that the sample should not be prewashed with aqueous solutions such as water or buffers as aldehyde markers are likely to be soluble in water and may be washed off the support if the sample is washed prior to treatment with the aldehyde detecting reagent (page 9, lines 29-34). Again, this is indicative of the fact that the presently claimed method is significantly different from the method of Shamsuddin, which is intended to detect a saccharide marker and not an aldehyde marker. In contrast, in the method of Shamsuddin, the sample is applied to a membrane filter and the membrane is then washed with deionized water for 1 minute then placed in Schiff's reagent for 1 minute (column 9, lines 14-15, column 10, lines 53-54). Shamsuddin also makes it clear that, although the procedure it describes is for rectal mucus samples, patients with positive results should be tested again using the same procedure with samples from other body sites, which can include breast fluid (, column 10, lines 24-32). Thus, it is quite clear from Shamsuddin that, whatever the sample used, the procedure should include a pre-washing step.

As, such the claims of the present invention are not anticipated by Shamsuddin, nor for the reasons stated above, obvious in light of the above. Withdrawal of this rejection is respectfully requested.

**Claim Rejections – 35 USC § 103**

The Examiner rejected claims 1-3, 5-8, 12, 14, 17, 23, 24, and 27 on the basis that a person skilled in the art would be motivated to treat a sample with Schiff's reagent without any pre-washing or wherein the sample of breast fluid is obtained from the nipple of one or both non-lactating breasts of the subject or a kit for performing the assay.

It should be noted that the Examiner has provided no support for the position that a person skilled in the art would be motivated to obtain a sample of breast fluid from the nipple of one or both non-lactating breast of a subject and treating it with a Schiff's reagent without any pre-washing and that this non-invasive and simpler method would work. If it was so obvious, then one would expect this simpler procedure to be the norm well before the date of the present invention.

For the reasons stated above, the methods of the present invention would not be obvious and a person skilled in the art would not be motivated to detect "aldehydes" in breast fluid without any pre-washing

Further, the Examiner similarly rejected the above-mentioned claims in light of Shamsuddin and Krepinsky et al. Applicant cannot agree.

First, it is stated that it certainly would not be obvious to a person skilled in the art why specimens of NAF from breast cancer patients would provide purple-magenta coloration without being previously treated with galactose oxidase (since only in this way an aldehyde group on Thomsen-Friedenreich Antigen could be created).

This invention cannot be anticipated from Krepinsky's teaching as applied to colorectal mucous either. One skilled in the art when applying Krepinsky's teaching to nipple aspirates would get negative results in all specimens even among those with breast cancer. Hence, a person with ordinary skills would logically conclude that there is no aldehyde, or plasmalogen in the NAF examined. A person with ordinary skill would abandon further search. Such a person would not expect that washing with water (i.e. prewashing) would be responsible for the negative test results.

A person reasonably skilled in the art would not expect that a plasmalogen, which is a lipid (or "fat" as cited by the Examiner) would be lost by washing with water that is the first step in the Krepinsky et al. process because: (i) lipids are insoluble or very poorly soluble in water, and (ii) they should stick to the polyester fabric; (iii) the water-washed specimens

were giving red color with Schiff reagent (from decomposition of Schiff reagent by basic substances) showing that such basic substances were retained by the fabric. Therefore, if no purple-magenta color is formed by the end of the procedure, it means that there is no lipid aldehyde or plasmalogen present in the breast secretion of a breast cancer patient.

Furthermore, the dye rosanilin (the colored component of Schiff reagent) is soluble in water, hence would not bind to the polyester fabric. A person reasonably skilled in the art would not anticipate that after treatment with the Schiff reagent, the reaction between the aldehyde marker and the Schiff reagent would form an insoluble product and hence adhere to the polyester fabric, leading to the tell-tale visual color change

Thus, being skilled in the art would lead to abandoning the trials rather than to further experimenting. The present inventors discovered that plasmalogen markers of the present invention behave differently from those in the colorectal cancer discovered by Krepinsky et al. This implies that they may be of different structure and thus of different physicochemical properties. Consequently, the development of a different procedure to detect these markers is not at all obvious and foreseeable from the prior art cited by the Examiner.

The Examiner states in reference to Krepinsky et al that one of ordinary skill in the art would have recognized that as it was known in the art that *water soluble, low molecular weight aldehydes derived from plasmalogens were known*...however, Applicant wishes to point out to the Examiner that there are no plasmalogens known from which low molecular weight aldehydes are derived. Support for the Examiner's statements are kindly requested.

Krepinsky, pages 13-14 teaches that more specific results are obtained when the method includes the step of washing with an aqueous phosphate buffer (Procedure A) than when no washing step is included (Procedure B) and even suggests that positive results from Procedure B should be re-tested using Procedure A.

In view of the teaching of both Shamsuddin and Krepinsky, it is particularly surprising that the present inventors have found that the washing of the sample before the application of an aldehyde-detecting reagent is actually detrimental to the procedure. This is made clear in the specification at page 3, line 29, page 5, lines 38-39 and page 9, lines 20-34. In addition, the following is a description of an experiment carried out by the inventors which demonstrates the problems which result from pre-washing the sample. The experiment refers to Example 2 of the patent specification and demonstrates that the

addition of a pre-washing step negates the results. This would certainly not have been expected from the teaching of either Shamsuddin or Krepinsky.

#### **Experimental**

Before the method described in Example 2 which does not include prewashing NAF specimens, the inventors cut one specimen into two halves and submitted one half to the method of Example 2 and the other half to the procedure including prewashing before the reaction with Schiff reagent. The half that was prewashed led to a negative result while the half that did not undergo prewashing led to a positive result. The clinical diagnosis of the woman was then obtained. She was diagnosed with cancer. The same procedure was performed on another specimen in which case both halves, one treated with prewashing and one without prewashing, provided negative results. The woman's clinical finding was that she was free of breast cancer. This confirmed that no prewashing is the proper procedure that would lead to the correct result.

As such, it is submitted that the claims of the present invention are not obvious in light of Shamsuddin and Krepinsky.

Last, the rejection by the Examiner of the above-noted claims in light of Sauter et al (1999) does not add anything to the Shamsuddin reference, as Sauter merely teaches that additional markers that are indicators of breast cancer can also be screened. It does not teach the methods of the present invention. Sauter only teaches that there are cancer markers in nipple aspirates. However, there are many cancer markers in NAF and other breast secretions already discovered. Nothing can be found in Sauter et al. that would lead to detecting cancer markers using the procedures of the present invention. In fact Sauter himself did not conclude that a simple application of Schiff reagent to nipple aspirate could reveal aldehyde cancer markers, he opted for an elaborated battery of tests for many other markers.

One would not have arrived at the present invention in light of Shamsuddin and Sauter. It is respectfully submitted that Sauter would not fill the deficiencies in Shamsuddin to enable a person skilled in the art to arrive at the teachings of the present invention.

Withdrawal of these rejections is respectfully requested.

#### **Summary**

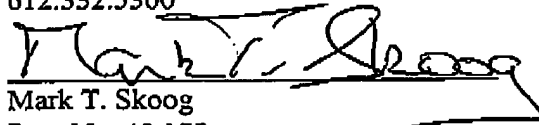
In summary, each of claims 1-3, 5-9, 12-14, 17-19, and 23-28 is in condition for allowance, and notification to that effect is earnestly solicited. The Examiner is invited to

contact the undersigned at the telephone listed below, if the Examiner believes that doing so will expedite prosecution of this application.

Respectfully submitted,

MERCHANT & GOULD P.C.  
P.O. Box 2903  
Minneapolis, Minnesota 55402-0903  
612.332.5300

Date: March 19, 2007

  
Mark T. Skoog  
Reg. No. 40,178  
MSkoog:jm